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(54) NOVEL GYMNEMIC ACID DERIVATIVES, PROCESS FOR THE PREPARATION THEREOF AND USE THEREOF AS MEDICINE

(57) This invention relates to new Gymnemic acid derivatives, their preparation, pharmaceutical composition or extract which contains them, and their medical

use, especially the use in the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation.

Description

The Invention Field

5 [0001] This invention relates to new Gymnemic acid derivatives, their preparation, pharmaceutical composition or extract which contains them, and their medical use, especially the use in the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation.

Background of technology

[0002] A lot of study on Gymnemic Acid derivatives have been done and all of these Gymnemic acid derivatives are from the plant called Gymnema cane, which is classified as *Gymnema sylvestre*. R. Br. And in India, it has been used to treat swelling, snake venom toxin, malaria, as a diuretic or to lower blood sugar level. Yet the Gymnemic acid derivatives and their biological activity mentioned in this invention haven't been reported up to date.

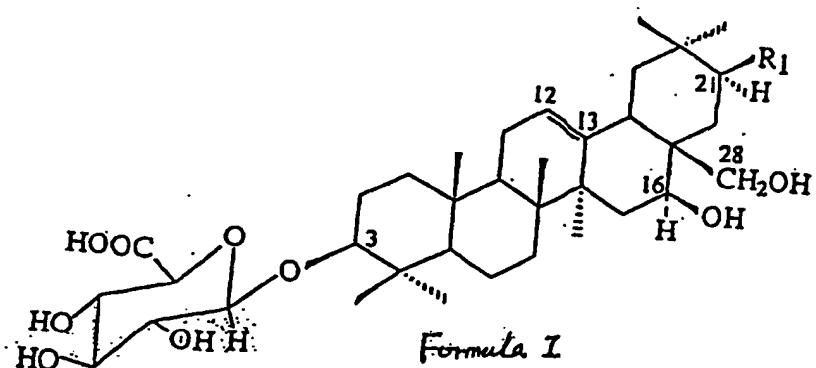
15 **The object of this invention**

[0003] The object of this invention is to find new Gymnemic acid derivatives and develop their medical use.

20 Description of this invention

[0004] This inventor has found out new Gymnemic acid derivatives formula I or II and further their medical use, especially in treating hyperglycemia, hyperlipidemia and platelets aggregation. The invention is now performed based on the discovery mentioned above.

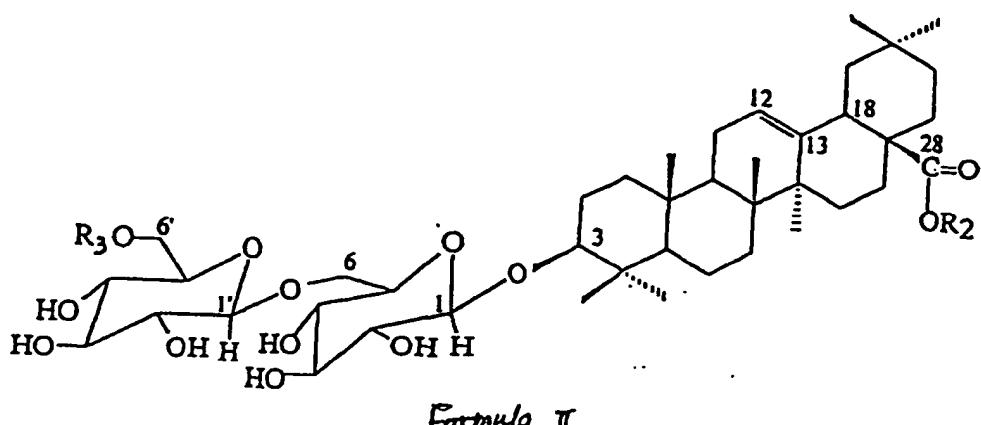
25 In the first part, this invention concerns Gymnemic Acid derivatives formula I or II,



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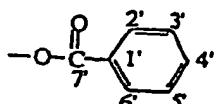
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wherein, R₁ is H or the radical represented by the following formula

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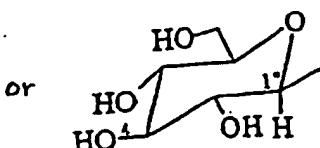
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R₃ is H, and R₂ symbolizes the following radical, or

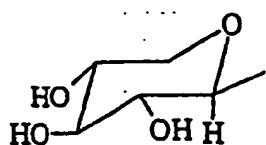
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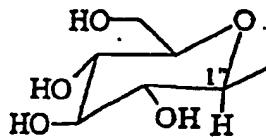
R₃ symbolizes the following radical,



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R₂ is H or the following radical,

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10 or pharmaceutically base addition salt thereof.

[0005] The second part of this invention relates to pharmaceutical composition which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as active ingredient, pharmaceutical carrier and excipient.

15 [0006] The third part of the invention involves Gymnemic Acid extract 12.5—40wt% of which is Gymnemic Acid derivative of formula I and/or II.

[0007] Another part of this invention relates to pharmaceutical composition for the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, pharmaceutical carrier and excipient.

20 [0008] Another part of the invention relates to a pharmaceutical composition for the prevention or treatment of diabetic, which includes at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, officinal carrier and excipient.

[0009] Another part of this invention relates to pharmaceutical composition for the prevention or treatment of higher blood lipid level, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, pharmaceutical carrier and excipient.

[0010] Another part of this invention relates to pharmaceutical composition for the prevention or treatment of platelets aggregation, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, pharmaceutical carrier and excipient.

30 [0011] Another part of this invention relates to the preparation of Gymnemic Acid derivative of formula I and II or pharmaceutical base addition salt thereof, which includes the following steps:

- a) extracting the plant Gymnema cane with ethanol under reflux and then concentrating;
- b) extracting concentrated liquid in step a) with cyclohexane, then extracting with n-butanol, concentrating to dryness under reduced pressure, and then obtaining a ointment;
- 35 c) subjecting the ointment in step b) to silica column chromatography with elute as chloroform: methanol=90:10-50:5 or 90:10-60:40, obtaining Gymnemic acid derivative of formula I and residue;
- d) subjecting the residue in step c) to C₁₈ column chromatography with elute as methanol/water (20/80-40/60), obtaining Gymnemic acid derivative of formula II;
- 40 e) if desired, converting the obtained Gymnemic acid derivative of formula I or II into pharmaceutical base addition salt with inorganic or organic base.

[0012] Another part of this invention relates to a method of preparation of the extract containing Gymnemic Acid derivative of formula I and II which range from 12.5-40wt%, which includes the following steps:

45 a) extracting Gymnema cane leaves with 60-95% ethanol and concentrating,
 b) extracting concentrated liquid in step a) with cyclohexane, then extracting with n-butanol, and then concentrating the extract under reduced pressure.

50 [0013] Another aspect of the invention relates to use of Gymnemic Acid derivative of formula I and II or the extract containing Gymnemic Acid derivative of formula I and II for the manufacture of medicament for the prevention or treatment of the diseases and conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation.

[0014] Finally, this invention relates to the method of preventing or treating the diseases and conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation, which includes administrating prophylactic or treatment effective quantity of Gymnemic Acid derivative of formula I and II to the patient suffered from diseases or conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation.

55 [0015] The term "patient" in the invention refers to mammal, including human being, and especially human being.

Detailed Description of the Invention

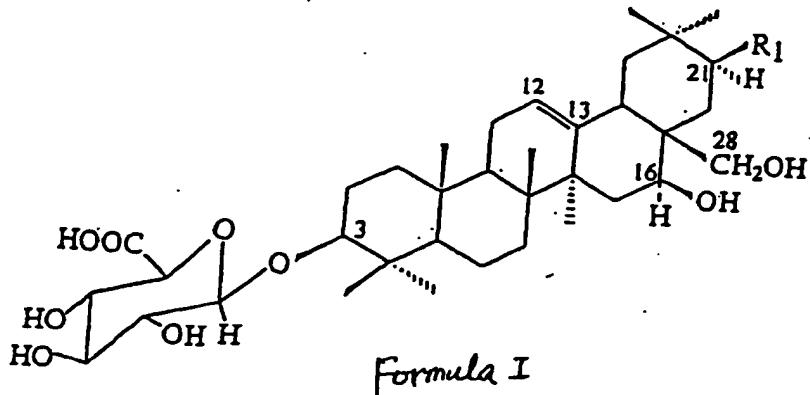
[0015] This invention relates to Gymnemic Acid derivative of formula I and II,

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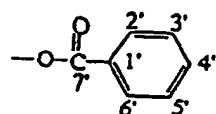
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Formula II

40 Wherein, R₁ is H or the group of the following formula

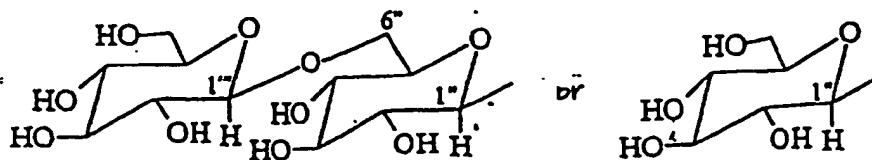
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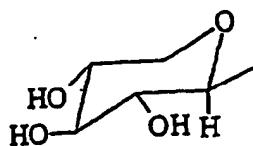
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R₃ is H, R₂ is the following group, or

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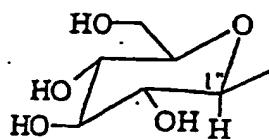


5 R_3 is the following group,



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R_2 is H or the following group,



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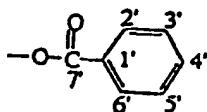
or pharmaceutical base addition salt.

25 According to the present invention, pharmaceutical base addition salt of Gymnemic acid of formula I or II includes a salt formed with pharmaceutical inorganic or organic base, inorganic base, for example, includes alkali or alkali earth metal hydroxide, alkali metal or alkali earth metal carbonate or bicarbonate, alkali metal may be selected from Li, Na, K, alkali earth metal may be selected from Ba, Mg, Ca etc. The organic base, for example, may be triethyl amine etc.

30 [0016] According to this invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula I wherein R_1 is H..

[0017] According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula I wherein R_1 as the following radical.

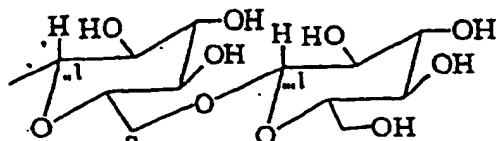
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According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula II wherein R_3 as H and R_2 as the following radical.

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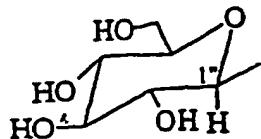


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According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula II wherein R_3 as H and R_2 as the following radical.

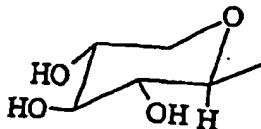
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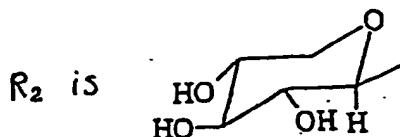
According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula II wherein
10 R_3 as the following radical and R_2 as H.

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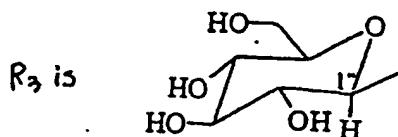
According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula II wherein
20 both R_3 and R_2 as the following radical respectively.

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According to this invention, the pharmaceutical composition mentioned here contains at least one kind of Gymnemic
40 Acid derivative of formula I and/or II and pharmaceutical carrier and excipient. For example, the pharmaceutical composition may include, for example, 1.25-2.10wt% compound A, 0.89-1.50wt% compound B, 2.40-3.80wt% compound C, 2.10-3.40wt% compound D, 2.74-4.60wt% compound E, and 3.24-5.40wt% compound F (compounds A, B, C, D, E and F as defined in examples below.). This pharmaceutical composition can be administrated by gastro intestine, parenteral or topical administration, such as oral, muscle, subcutaneous, peritonaeum, vein etc. The forms of drug suitable for intestine administration are for example tablet, capsule, solution, suspension, powder, granulate etc. The forms of drug suitable for parenteral include injection solution, frozen dry powder for injection etc. The drug forms suitable for the topical are for example, ointment, cream, paste, patch, and spray. Of all these forms, oral administration is preferred while capsule is preferred in oral form. The pharmaceutical carrier or excipient of the pharmaceutical composition includes binding agent, filling material, wet agent, desintegrate agent, surfactant, lubricating agent, dilut agent etc. If desired, colour agent, flavoring agent, solubilizer, buffer etc are also used. The diluting agents in the invention include starch, dextrin, lactose, microcellulose, silica gel, etc. And silica gel is preferred. The wetting agents includes water and ethanol, lubricating agents include talcum powder, stearic magnesium.

[0018] The pharmaceutical composition in the present invention can be produced by the known method in this art. For example, mix Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt with pharmaceutical carrier and excipient.

[0019] The dos of Gymnemic Acid derivative of formula I and II depends on many factors such as the characters and seriousness level of the disease to be prevented or treated, sex, age, weight, individual response, specific compound, administration route and times of administration. Generally the specific dose depends on the judgment of phy-

sician. Generally speaking, the dosage of pharmaceutical composition Gymnemic Acid derivative of formula I and II can be in the form of single dose and taken 1-4 times per day.

According to this invention, the pharmaceutical base said of formula I Gymnemic Acid derivative can be prepared as follows:

5 a) crushing dry leaves of *Gymnema* cane, then extracting three times with 60-95% ethanol under reflux, two hours for each, combining extracted liquid and concentrating under reduced pressure until there was no ethanol, for use;

10 b) extracting the concentrated mixtures in step a) for 3 to 6 times with cyclohexane, then extracting with n-butanol, concentrating to dryness under reduced pressure, obtaining dry extract, ready for use;

15 c) subjecting the dry extracts in step b) to silica gel column chromatography with elute as mixture of chloroform and methanol at the ratio 90:10 to 60:40, obtaining derivatives of formula I,

20 d) If desired, converting the derivative of formula I in step c) into pharmaceutical base salt thereof.

According to this invention, the Gymnemic Acid derivative of formula II can be prepared as follows:

15 a) Crushing dry leaves of *Gymnema* cane, then extracting three times with 60-95% ethanol under reflux, two hours for each, combining extracted liquid and concentrating under reduced pressure until there was no ethanol, ready for use.

20 b) extracting concentrated mixtures for 3 to 6 times with cyclohexane, then extracting with n-butanol; concentrating to dryness under reduced pressure ready for use;

25 c) mixing the dry extracts in step b) with raw silica gel; subjecting separation with thin layer chromatography of silica gel H; the mixture of chloroform and methanol at the ratio 90:10 to 50:50 as elute, subjecting the residue after elute to C₁₈ column chromatography with elute as methanol/water (20:80-40:60), obtaining derivative of formula II;

30 d) if desired, converting the derivative of formula II in step c) into pharmaceutical base salt thereof.

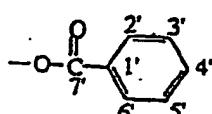
[0020] According to this invention, the extract products with 12.5-40 wt% Gymnemic Acid derivative of formula I and formula II can be prepared as follows: raw powder of *Gymnema* cane leaves were refluxed 1-4 times with 60-95% ethanol, the amount of solvent for each is 6ml/g, extract time is 1-3 hours. The extract mixtures were combined together and distilled under reduced pressure till there was no ethanol, the concentrated mixture was extracted with cyclohexane for 1-3 times, 500ml solvents was used for each time. Then the mixture was extracted for 1-3 times 500ml with n-butanol, all the extract mixtures were combined and distilled under reduced pressure to obtain the desired product.

This invention gives a further illustration by the preparation examples and biological active experiment, but it does not mean any limitation to the invention.

35 Example 1

[0021] Preparation of compound A (Gymnemic Acid derivative of formula I wherein the R₁ being H) and compound B (Gymnemic Acid derivative of formula I wherein the R₁ being group as follow)

40



[0022] 1000g raw powder of *Gymnema* cane leaves were refluxed for 3 times with 60% ethanol, 6L solvents were used for each, and 2 hours for each time. The extract mixtures were combined together and distilled under reduced pressure till there was no ethanol, the concentrated mixture was extracted with 0.5L cyclohexane and butane for 3 times. All the n-butane extract mixtures were combined and distilled under reduced pressure to obtain 64.0g dry extract product. 32.0g dry extract substance was added into 60g 60-100 mesh rough silica gel, the mixture was vaporized to dryness on a water pan. 450g 200-300 mesh (m) silica gel were loaded into column by a wet method. then treated sample was added to be subjected to column separation with elute as 90:10-60:40 of mixtures of chloroform and methanol mixtures, 80mg compound A and 60mg compound B were obtained.

55 The physics and chemistry data of compound A and compound B were showed as follows:

Compound A:

5 Amorphous powder: mp198 - 202°C; $[\alpha]_{20}^D +16.0^\circ$ (c0.10, M OH); IR ν_{max} 3414 (OH), 1724 (COOH), 1636 (C=C), 1458, 1380, 1054cm⁻¹; ¹HNMR (500MHz, pyridin - d₅) 80.86 (3H, s, Me), 0.95 (3H, s, Me), 1.01 (9H, s, 3 \times Me), 1.32 (3H, s, Me), 1.39 (3H, s, Me), 3.39(1H, dd, J=4.3 and 11.8Hz, H - 3 α), 3.68(1H, d, J=10.5Hz, H - 28a), 4.43 (1H, d, J=10.5Hz, H - 28b), 4.68 (1H, m, H - 16 α), 5.04 (1H, d, J=7.8Hz, H - 1 of gluconic acid), 5.26 (1H, brs, H - 12); ¹³CNMR (125MHz, pyridin - d₅), See table 1 and 2; FAB MSm/z 657[M+Na]⁺.

Compound B:

10 Amorphous; mp192 - 195°C; $[\alpha]_{20}^D +27.2^\circ$ (c 0.15, MeOH); IR ν_{max} 3444 (OH), 1724, 1700, 1635(C=C), 1457, 1388, 1280, 1074, 720cm⁻¹; ¹HNMR (500MHz, pyridin) 80.98 (3H, s, Me), 1.01(3H, s, Me), 1.02(9H, s, 3 \times Me), 1.07(3H, s, Me), 1.30(3H, s, Me), 1.34(3H, s, Me), 1.36 (3H, s, Me), 3.40 (1H, dd, J=4.5 and 12.0Hz, H - 3 α), 3.70 (1H, d, J=10.2Hz, H-28a), 4.42 (1H, d, J=10.2Hz, H-28b), 4.70(1H, m, H - 16 α), 5.10(1H, d, J=7.8Hz, H - 1 of gluconic acid), 5.70 (1H, dd, J=4.7 and 12.3Hz, H - 21 α), 7.47 (3H, overlap, H - 3', - 4' and - 5'), 8.25(2H, dd, J=1.4 and 4.8Hz, H - 2' and - 6'); ¹³CNMR (125MHz, pyridin - d₅), See table 1 and 2 ; FAB MSm/z 777[M+Na]⁺.

Table 1:

13CNMR data of glucoside liquid of compound A and B			
	Carbon atom	Compound A	Compound B
15	1	38.8	38.8
20	2	26.6	26.6
25	3	89.0	89.0
30	4	39.5	39.6
35	5	55.7	55.7
40	6	18.4	18.4
45	7	32.9	33.0
50	8	40.1	40.1
55	9	47.1	47.1
	10	36.7	36.7
	11	23.8	23.9
	12	122.6	123.1
	13	143.9	142.6
	14	43.8	43.7
	15	36.7	36.8
	16	66.6	66.4
	17	41.1	43.8
	18	44.4	44.2
	19	47.1	47.2
	20	31.1	36.0
	21	34.3	75.6
	22	26.2	33.3
	23	28.2	28.2
	24	16.9	16.9
	25	15.7	15.7
	26	17.0	17.0
	27	27.2	27.0
	28	68.9	66.8

Table 1: (continued)

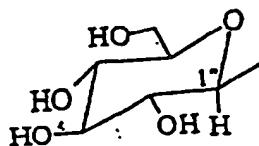
¹³ CNMR data of glucoside liquid of compound A and B		
Carbon atom	Compound A	Compound B
29	33.4	29.2
30	24.1	18.8
Acyl 1'		131.6
Acyl 2'		129.9
Acyl 3'		128.9
Acyl 4'		133.2
Acyl 5'		128.9
Acyl 6'		129.9
Acyl 7'		166.3

Table 2:

¹³ CNMR data of saccharic part compound A and B		
Carbon atom of C - 3	Compound A	Compound B
Glutamic acid 1	107.3	107.3
Glutamic acid 2	75.6	75.6
Glutamic acid 3	78.2	78.2
Glutamic acid 4	73.5	73.6
Glutamic acid 5	77.8	77.7
Glutamic acid 6	173.1	173.3

Example 2:

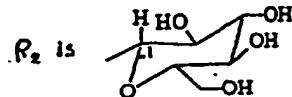
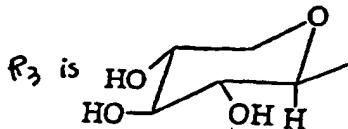
[0023] Preparation of Compound C (formula II Gymnemic Acid derivative with R₃ as H and R₂ as follow group),



compound D (formula II Gymnemic Acid derivative with R₃ as follows and R₂ as H),



compound E (formula II Gymnemic Acid derivative with R₃ as follow),



10 R_2 as follow and compound F(formula II Gymnemic Acid derivative with R_3 as H and R_2 as follow)



20 [0024] 1000g raw powder of Gymnema cane leaves were refluxed for 3 times with 75% ethanol. 6.0L solvents were used, 2 hours for each time. The extract mixtures were combined together and distilled under reduced pressure till there was no ethanol, the condensed mixture was extracted with 0.5L cyclohexane and butane for 3 times. All the n-butane extract mixtures were gathered and distilled under reduced pressure to obtain 72.0g dry extract product. 36.0g dry extract substance was taken and added into 60g 60-100 mesh rough silica gel, the mixture was vaporized to dryness on a water pan, 400g 200-400 mu silica gel H used as thin-layer separation were loaded into column in a wet method, then treated sample was added undergoing column separation with elute as 90:10-50:50 chloroform-methanol mixtures, 130mg compound C, 115mg compound D, 160mg compound E and 195mg compound F were obtained respectively.

25 The physics and chemistry data of compound C were shown as follows:

30 [0025] Amorphous powder; mp206 - 209°C; $[\alpha]_{20}^D$ - 16.0° (c 0.11, MeOH); IR_{max} 3424(OH) 1735(COOR), 1636(C=C), 1457, 1034cm⁻¹; ¹HNMR (400MHz, pyridin - d₅) 80.82 (3H, s, Me), 0.87 (3H, s, Me), 0.91 (3H, s, Me), 0.97 (3H, s, Me), 1.07(3H, s, Me), 1.20 (3H, s, Me), 1.23 (3H, s, Me), 3.17 (1H, dd, J=3.5 and 10.2Hz, H- 18), 3.30(1H, d, J=3.9 and 11.7Hz, H- 3 α), 5.37(1H, brs, H- 12), ¹³CNMR (100MHz, pyridin - d₅), See table 3 and 4; FAB MS_{m/z} 943[M+H]⁺.

The physics and chemistry data of compound D were shown as follows:

35 [0026] Amorphous powder; mp202 - 204°C ; $[\alpha]_{20}^D$ - 3.2° (c 0.15, MeOH); IR_{max} 3410 (OH), 1710(COOR), 1638(C=C), 1458, 1036cm⁻¹; ¹HNMR (400MHz, pyridin - d₅) 80.87 (3H, s, Me), 0.91 (3H, s, Me), 0.96 (3H, s, Me), 1.02(3H, s, Me), 1.10(3H, s, Me), 1.24(3H, s, Me), 1.29 (3H, s, Me), 3.30 (1H, dd, J=4.5 and 11.5Hz, H- 3 α), 5.38(1H, brs, H- 12), ¹³CNMR(100MHz, pyridin - d₅), See table 3 and 4; FAB MS_{m/z} 935[M+Na]⁺.

40 The physics and chemistry data of compound E were shown as follows:

45 [0027] Amorphous powder; mp212 - 215°C; $[\alpha]_{20}^D$ - 9.6° (c 0.20, MeOH); IR_{max} 3414 (OH), 1740 (COOR), 1636(C=C), 1460, 1364, 1044, 896cm⁻¹; ¹HNMR (500MHz, pyridin - d₅) 80.85 (3H, s, Me), 0.90 (3H, s, Me), 0.94 (3H, s, Me), 1.00(3H, s, Me), 3.19 (1H, dd, J=4.0 and 13.7Hz, H- 18), 3.32(1H, d, J=4.4 and 11.7Hz, H- 3 α), 5.40(1H, brs, H- 12), ¹³CNMR (100MHz, pyridin - d₅), See table 3 and 4; FAB MS_{m/z} 943[M+Na]⁺.

50 The physics and chemistry data of compound F were shown as follows:

55 [0028] Amorphous powder; mp209 - 211 °C ; $[\alpha]_{20}^D$ - 12.1° (c 0.12, MeOH); IR_{max} 3424 (OH), 1734 (COOR), 1636(C=C), 1458, 1047cm⁻¹; ¹HNMR (400MHz, pyridin - d₅) 80.87 (3H, s, Me), 0.90 (3H, s, Me), 0.92 (3H, s, Me), 1.00(3H, s, Me), 1.09(3H, s, Me), 1.22(3H, s, Me), 1.26 (3H, s, Me), 3.20 (1H, dd, J=3.5 and 13.6Hz, H- 18), 3.33(1H, d, J=4.4 and 11.5Hz, H- 3 α), 5.39(1H, brs, H-12), ¹³CNMR (100MHz, pyridin - d₅), See table 3 and 4; FAB MS_{m/z} 1127[M+H]⁺.

Table3:

13CNMR data of glucoside ligand of compound C-F				
Carbon atom	Compound C	Compound D	Compound E	Compound F
1	38.8	38.7	38.7	38.7

Table3: (continued)

13CNMR data of glucoside ligand of compound C-F					
	Carbon atom	Compound C	Compound D	Compound E	Compound F
5	2	26.6	26.7	26.7	26.7
10	3	88.9	89.0	89.0	89.0
15	4	39.4	39.5	39.5	39.5
20	5	55.7	55.8	55.8	55.8
25	6	18.4	18.3	18.5	18.5
30	7	33.0	33.1	33.1	33.1
35	8	39.8	39.9	39.9	39.9
40	9	47.9	48.0	48.0	48.0
45	10	36.9	37.0	37.0	37.0
	11	23.7	23.7	23.8	23.7
	12	122.9	122.8	123.0	122.9
	13	144.0	144.4	144.0	144.1
	14	42.0	42.1	42.1	42.1
	15	28.2	28.2	28.2	28.2
	16	23.3	23.4	23.4	23.4
	17	46.9	46.5	47.0	47.0
	18	41.6	41.9	41.7	41.7
	19	46.2	46.1	46.2	46.3
	20	30.7	30.9	30.8	30.8
	21	33.9	34.4	34.0	34.0
	22	32.5	33.1	32.5	32.5
	23	28.1	28.2	28.2	28.3
	24	17.0	17.0	17.0	17.0
	25	15.5	15.8	15.6	15.6
	26	17.4	17.3	17.5	17.5
	27	26.0	26.1	26.1	26.1
	28	176.4	180.2	176.5	176.5
	29	33.1	33.2	33.2	33.2
	30	23.6	23.7	23.7	23.7

Table 4:

13CNMR data of saccharic part of compound C-F				
C - 3	Compound C	Compound D	Compound E	Compound F
Glc1	106.9	107.0	107.0	106.9
Glc2	75.1	75.0	75.0	75.2
Glc3	78.4	78.3	78.3	78.4
Glc4	71.6	71.5	71.5	71.5

Table 4: (continued)

13CNMR data of saccharic part of compound C-F					
	C - 3	Compound C	Compound D	Compound E	Compound F
5	Glc5	77.0	77.0	77.0	77.0
	Glc6	70.4	70.4	70.4	70.5
10	Glc'1	105.4	105.4	105.4	105.4
	Glc'2	75.5	75.6	75.6	75.6
	Glc'3	78.5	78.5	78.5	78.6
15	Glc'4	71.7	71.6	71.6	71.7
	Glc'5	78.4	76.9	76.9	78.5
	Glc'6	62.7	69.8	69.8	62.6
20	Xyl1		106.0	106.0	
	Xyl2		74.9	74.9	
	Xyl3		78.0	78.1	
	Xyl4		71.1	71.1	
25	Xyl5		67.0	67.1	
	C-28				
	Glc"1	95.7		95.8	95.7
	Glc"2	74.1		74.1	73.9
30	Glc"3	78.8		78.9	78.7
	Glc"4	71.0		71.1	70.9
	Glc"5	79.3		79.3	78.0
35	Glc"6	62.1		62.2	69.3
	Glc""1				105.3
	Glc""2				75.2
	Glc""3				78.5
40	Glc""4				71.7
	Glc""5				78.4
	Glc""6				62.7

Biological active experiments

45 Experiment example 1

[0029] Effect of compound B on the increasing blood sugar in rats caused by sucrose

50 [0030] Female SD rats fasted for 24 hours were divided into several groups randomly. Test groups are given 50,100,200mg/kg compound B, the positive-control group was given 100mg/kg phenformin, The normal group , control group and blank group were given the same amount of water, the given medicine volume was 10mg/kg, after 30 minutes each group was given saccharose 1/kg(5ml/kg) except normal group, and blood was extracted from the eyes of rats after 30, 60 and 120 minutes respectively, the content of glucose in the serum was measured.

55 The result was, after the rats were given saccharose for 30 ,60 minutes, the value of blood sugar increased apparently. The compound B 200mg/kg and phenformin 100mg/kg within 30 minutes can both reduce the increased value of blood sugar remarkably, and the strength of the two compounds was similar. Results see table 5.

Table 5:

The effect of compound B on the increasing blood sugar in rats caused by sucrose (X±SD, n=10)				
group	dose (mg/kg)	Value of blood sugar (mmol/L)		
		30minutes	60 minutes	120 minutes
Normal group		3.56±0.64	4.12±0.72	3.76±0.69
control group		6.58±0.87 ^{ΔΔ}	5.93±1.27 ^{ΔΔ}	4.54±1.37
compound B	50	6.03±0.86	6.42±0.78	4.26±1.03
	100	5.12±1.29 ^{**}	5.77±1.09	4.53±0.94
	200	4.43±0.72 ^{**}	4.73±0.83 ^{**}	4.07±0.70
phenformin	100	4.24±0.87 ^{**}	4.74±0.90 [*]	4.79±1.03

^{ΔΔ}P<0.01, compared with normal group;^{*}P<0.05,^{**}P<0.01, compared with control group.

20

Experiment example 2

[0031] The effect of compound B on the contents of TG, cholesterol in serum of hyperlipidemia rats

Male SD rats with the weight of 130-170g, normal group was given common food, other groups were given food having high lipid content (1%cholesterol, 10%lard, 0.3% cholic acid, 0.2% methylthio imidazole and 88.5% common forage, made into block.). In sequentially 14 days, rats fasted 12 hours were measures by reagent box method to obtain the contents of TG and cholesterol in serum. Then they were divided according to the value of blood grease into different group. The experimental group was given 50, 100, 200mg/kg compound B, the positive-control group was given clofibrate 100mg/kg, the control group was given water, the given medicine volume was 10ml/kg, for 10 days, each group was still given high fat forage for 5 days before given medicine, common forage was given in the later 5 days, the rats were fasted for 11 hours before being given the final administration and blood of rat were extracted to obtain the content of TG and cholesterol in serum 1 hour after being given medicine.

The results show that 10 days after rats were given forage having high grease, the contents of TG and cholesterol increased apparently, compound B 50, 100, 200mg/kg and clofibrate 100mg/kg can both reduced the contents of TG and cholesterol in blood serum of hyperlipidemia rats, compound B 200mg/kg have the same effect as 100mg/kg clofibrate in reducing hyperlipidemia, see table 6

Table 6:

The effect of compound B on the contents of blood lipid in hyperlipidemia rats (X±SD, n=9 - 10)					
group	dose (mg/kg)	TG (mmol/L)		Total cholesterol (mmol/L)	
		Before administration	After administration	Before administration	After administration
Normal group		1.02±0.22	1.04±0.15	2.43±0.41	1.99±0.47
control group		2.64±0.82	3.04±0.93	4.10±0.51 ^{ΔΔ}	4.77±0.63 ^{ΔΔ}
compound B	50	2.72±0.61	2.41±0.44	4.29±0.60	3.92±0.58 ^{**}
	100	2.54±0.90	1.75±0.53 ^{**}	4.02±0.59	2.94±0.66 ^{**}
	200	2.72±0.76	1.37±0.40 ^{**}	4.18±0.61	2.31±0.74 ^{**}
clofibrate	100	2.51±0.77	2.72±0.74	4.33±0.51	2.15±0.76 ^{**}

^{ΔΔ}P<0.01, compared with normal group;^{**}P<0.01, compared with control group

55

Experiment example 3

[0032] Effect of compound B on blood platelet aggregation in rabbits invitro.

5 Taken blood from rabbit heart with puncture, 3.8 % potassium citrate for anticoagulation (1:9), centrifuge 15 minute in 1000rpm, take upper layer as rich blood platelet plasma (prp), And then centrifuge 10 minutes with 4000rpm, take supernatant as poor blood platelet plasma (ppp). Transfer PPP200ul to nephelotube, and add into different concentration physiological brine solution 10ul of the compound B, final concentration is respectively 250,500,1000 μ g/ml, add physiological brine 10 μ l of aspirin to positive control tube ,put it into measuring cell after warming for 2 minutes in 37°C, and add into physiological brine solution10ul of ADP sodium salt with stirring, final concentration is 1.0×10^{-5} M. Observe 10 the maximal aggregation ratio on PAM-1 type of blood platelet instrument within 3 minutes.

[0033] The result shows that the compound B 500,1000 μ g/ml and aspirin 250 μ g/ml obviously inhibit blood platelet from aggregating.

Table 7:

Effect of compound B on blood platelet aggregation in rabbit (X \pm SD, n=8)				
Group	Final concentration	Maximal aggregation ratio (%)	Inhibition ratio	
control group		47.9 \pm 5.2		
compound B	250	43.6 \pm 7.0	9.0	
	500	35.9 \pm 4.5**	25.1	
	1000	27.8 \pm 4.8**	42.0	
Aspirin	250	23.7 \pm 6.0**	50.3	

25 **P<0.01,compared with control group

Experiment example 4

[0034] Effect of compound F on blood sugar elevation in rat.

30 Male Kun Ming strain mice are divided into randomly experiment groups, they respectively take orally the compound F 50,100,2000mg/kg, positive control group respectively take orally glybenclamide 50mg/kg, blank control group and normal control group take orally the same distilled water, the volume of medicine given is 20ml/kg, lasting 7 days. They are forbidden to give feedstuff 10 hours before the last time of administration. Each group is given dextrose 35 solution 2.5g/kg (10ml/kg) except of normal control group. Before and after 30 minute of administration of dextrose, pick blood 100ul from eyepit, measure the content of dextrose in serum according to the way of dextrose oxygenation enzyme.

35 Result, after mice take orally dextrose 30 minutes , blood sugar obviously rise, Both the compound F 100,200mg/kg and 50mg/kg obviously inhibit blood sugar in mice from rising. The function of the compound B 200mg/kg and glybenclamide 500mg/kg lowering blood sugar is similar, which may be seen in table 8.

Table 8

Group		Dose (mg/kg)	Value of blood sugar
		0 minute	30 minutes
Normal group		6.20 \pm 1.01	6.64 \pm 1.04
control Group		6.55 \pm 1.16	13.94 \pm 3.22
compound B	50	6.79 \pm 1.16	12.01 \pm 1.88
	100	6.09 \pm 1.34	9.59 \pm 2.25**
	200	6.42 \pm 0.99	9.16 \pm 1.08**
glybenclamide	50	4.48 \pm 0.83**	8.18 \pm 1.72**

45 P<0.01,compared with normal group:
55 **p<0.01,compared with control group

Experiment example 5

[0035] Effect of compound F on the content of triglycerides and cholesterol in the serum of hyperlipidemia rat.

[0036] Male SD rat with weight of 130-170g. The normal group is given general feedstuff, and the other groups are given high-fat (1% cholesterol, 10% pig oil, 0.3% clolic acid, 0.2% methylthio imidazole and 88.5% normal feedstuff are made stuff by oneself). After the feedstuff is in run for 14 days and the mice rat is forbidden to eat for 12 hours, measure the content of triglycerides and cholesterol in rat's serum. And then the rat are grouped randomly according to blood lipid value. The experiment group is given to this compound F 50,100,200mg/kg., the ositive-control group is given orally to clofibrate of 100mg/kg, the control group is given distilled water. The volumn of administration is given 10ml/kg, lasting 10 days. Each group is given high-fat feedstuff in the former 5days of giving drugs, and then general feedstuff in the next 5days. Fasting of 11 hours is conducted before the last time of giving drugs. After giving drugs for 1 hour, take blood and measure the content of ester and cholesterol in the blood serum..

Result

[0037] The content of TG and cholesterol in the blood serum of rat elevates obviously after given high-fat feedstuff for 10 days. 50mg/kg, 100mg/kg, 200mg/kg of compound F and 200mg/kg clofibrate make the level of triglycerides and cholesterol in blood serum of rat with high-fat blood diseases lower. The action 200mg/kg of the compound F is the similar as to that of 100mg/kg of clofibrate in the function of lowering blood fat.(table 9)

Table 9:

the effect of compound B on the content of blood fat of rat with high-fat blood disease. (X±SD, n=9-10)					
group	dose (mg/kg)	triglycerides (mmol/L)		Total cholesterol (mmol/L)	
		Before administration	After administration	Before administration	After administration
Normal group		1.02±0.22	1.04±0.15	2.43±0.41	1.99±0.47
Control group		2.64±0.82	3.04±0.93	4.10±0.51 ^{△△}	4.77±0.63 ^{△△}
compound B	50	2.72±0.61	2.41±0.44	4.29±0.60	3.92±0.58 ^{**}
	100	2.54±0.90	1.75±0.53 ^{**}	4.02±0.59	2.94±0.66 ^{**}
	200	2.72±0.76	1.37±0.40 ^{**}	4.18±0.61	2.31±0.74 ^{**}
clofibrate	100	2.51±0.77	2.72±0.74	4.33±0.51	2.15±0.76 ^{**}

△P<0.01, (compared with normal group);
**P<0.01 (compared with control group)

Experimental example 6 effect of compound B affects on blood platelet aggregation in rabbit.

[0038] Take blood from rabbit heart by puncturing, 3.8% of potassium citrate for anticoagulation (1:9), centrifuging 15 minute in 1000rpm, take the upper layer as blood platelet rich plasma(prp), And then centrifuguga 10 minute in 4000rpm, take supernatant as blood platelet poor plasma(ppp). the final concentration of compound B is revetively 250,500,1000 μ g/ml, final concentration is respectively 250,500,1000ug/ml, add 10ul of physical brine of aspirin to the positive-control tube to final concentration of 250 μ g/ml, add 10ul of physical brine to control tube to the final concentration of 250 μ g/ml. Observe the maximal aggregation ratio on PAM-1type instrument of blood platelet aggregation within 3 minute.

The result shows that 500,1000μg/ml of the compound F and aspirin 250μg/ml obviously inhibit the aggregation of blood platelet.

Table 10:

the effect of the compound F on aggregation of rabbit's blood platelets in vitro.			
group	Final concentration (μg/ml)	Maximal aggregation rate (%)	Inhibition rate (%)
control		47.9±5.2	

Table 10: (continued)

the effect of the compound F on aggregation of rabbit's blood platelets in vitro.			
group	Final concentration ($\mu\text{g/ml}$)	Maximal aggregation rate (%)	Inhibition rate (%)
compound F	250	43.6 \pm 7.0	9.0
	500	35.9 \pm 4.5**	25.1
	1000	27.8 \pm 4.8**	42.0
aspirin	250	23.7 \pm 6.0**	50.3

**P<0.01(compared with the control)

Experimental example 7

[0039] Effect of compound B on blood sugar in normal mice.

[0040] Male Kun Ming strain mice are divided into randomly experiment groups, they respectively take orally the compound B 50,100,2000mg/kg, positive control group respectively take orally tolbutol 100mg/kg, blank control group takes orally the same distilled water, the volume of medicine given is 20ml/kg, lasting 14 days. They are administrated to test drug (provided that they are pre-forbidden to give food 5 hrs before administration) after the days 1, 3, 7, 14 of administration. After 3hrs of administration, pick blood 10ul from eyepit, measure the content of dextrose in serum according to the way of reagent box.

Result shows that compound B 50, 100, 200mg/kg for continuely administration 14 days has no obvious effect on blood sugar of normal mice, but tolbutol starting from day 3 of administration show obvious effect for lowering the blood sugar of normal mice. Result is also seen in table 11.

Table 11,

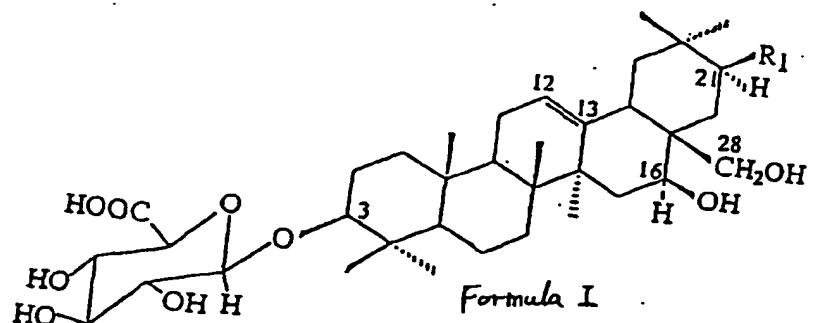
Effect of compound B on blood sugar in normal mice. ($\bar{X}\pm\text{SD}$, n=10)					
Group	Dose (mg/kg)	Value of blood sugar			
		1	3	7	14 (day)
control Group		5.21 \pm 1.10	7.10 \pm 1.30	8.56 \pm 0.74	7.52 \pm 1.29
compound B	50	5.84 \pm 0.94	7.56 \pm 0.92	8.51 \pm 1.06	8.27 \pm 0.66
	100	6.48 \pm 1.28	7.73 \pm 2.26	8.71 \pm 0.97	7.45 \pm 1.59
	200	6.41 \pm 1.04	6.28 \pm 1.19	8.46 \pm 0.88	7.86 \pm 1.56
tolbutol	100	6.48 \pm 1.18	5.22 \pm 0.80**	6.62 \pm 0.96	5.75 \pm 1.02

P<0.01,compared with normal group:

**p<0.01,compared with control group

Claims

45 1. Gymnemic Acid derivative of general formula I or general formula II,

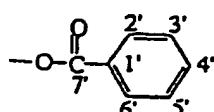




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wherein, R₁ is H or the radical represented by the following formula

20

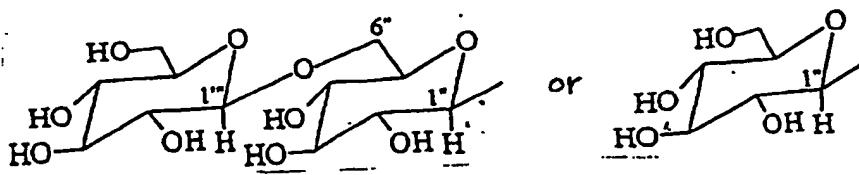


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R_3 is H, and R_2 symbolizes the following radical, or

25

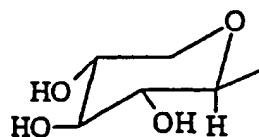
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R₃ symbolizes the following radical,

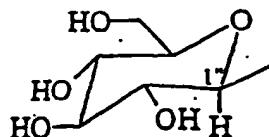
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B_6 is H or the following radical

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50



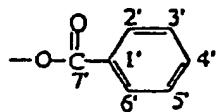
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or pharmaceutically base addition salt thereof.

2. Gymnemic Acid derivatives of claim 1 wherein R₁ in formula I is hydrogen.

3. Gymnemic Acid derivatives of claim 1, wherein R₁ in formula I is a group of the formula:

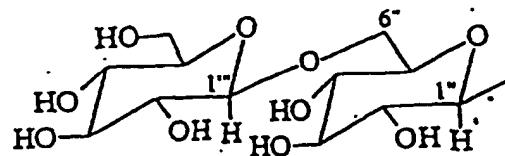
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4. Gymnemic Acid derivatives of claim 1, wherein R₃ in formula II is hydrogen, R₂ is group of formula:

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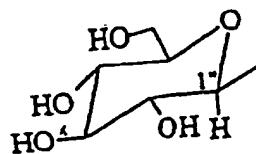
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5. Gymnemic Acid derivatives of claim 1, wherein R₃ in formula II is hydrogen, R₂ is group of formula:

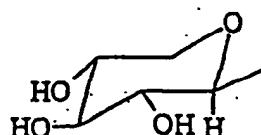
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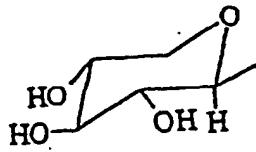
30 6. Gymnemic Acid derivatives of claim 1, wherein R₂ in formula II is hydrogen, R₃ is group of formula:

35



40 7. Gymnemic Acid derivatives of claim 1, wherein R₃ in formula II is group of formula

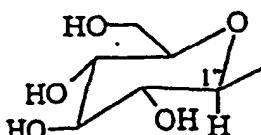
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R₂ is group of formula:

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8. Pharmaceutical composition which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as active ingredient, pharmaceutical carrier and excipient.

5 9. Pharmaceutical composition for the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, pharmaceutical carrier and excipient.

10 10. A composition of claims 1 or 2,which contains Gymnemic Acid derivative of formula I and/or II ,wherein based on the weighe of the composition,the content of compounds A,B,C,D,E and is 1.25-2.10% compound A, 0.89-1.50% compound B, 2.40-3.80% compound C, 2.10-3.40% compound D, 2.74-4.60% compound E and 3.24-5.40% compound F.

15 11. A extract of *Gymnema sylevestre*.R.Br which contains 12.5-40wt% Gymnemic acid derivatives of formula I and formula II.

12. Use of Gymnemic Acid derivative of formula I and II or the extract containing Gymnemic Acid derivative of formula I and II for the manufacture of medicament for the prevention or treatment of the diseases and conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation.

20 13. A method of the preparation of Gymnemic Acid derivative of formula I and II or pharmaceutical base addition salt thereof, which includes the following steps:

25 c) extracting the plant *Gymnema* cane with ethanol under reflux and then concentrating;

d) extracting concentrated liquid in step a) with cyclohexane , then extracting with n-butanol, concentrating to dryness under reduced pressure, and then obtaining a ointment;

c) subjecting the ointment in step b) to silica column chromatography with elute as chloroform: methanol=90:10-50:5 or 90:10-60:40, obtaining Gymnemic acid derivative of formula I and residue;

d) subjecting the residue in step c) to C₁₈ column chromatography with elute as methanol/water (20/80-40/60), obtaining Gymnemic acid derivative of formula II;

30 e) if desired, converting the obtained Gymnemic acid derivative of formula I or II into pharmaceutical base addition salt with inorganic or organic base.

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INTERNATIONAL SEARCH REPORT		International application No. PCT/CN00/00010
A. CLASSIFICATION OF SUBJECT MATTER C07H15/256, C07J63/00, A61K31/70, A61K35/78 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED C07H15/256, C07J63/00, A61K35/78, A61K31/70 Minimum documentation searched (classification system followed by classification symbols) IPC7 C07H15/256, C07J63/00, A61K31/70, A61K35/78		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched WPI, EPODOC, PAJ, CAPLUS, Gymnemic acid, derivative, and its structures		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Chinese Patent Documents		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US-A-5843909	1-13
Y	EP-A1-0406516	1-13
A	Chem. Pharm. Bull. 37(3)852-854, 1989	1-7,13
A	Tetrahedron Letters Vol. 30 No. 12, pp 1547-1550, 1989	1-7,13
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
<ul style="list-style-type: none"> • Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "I" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed 		
<ul style="list-style-type: none"> "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family 		
Date of the actual completion of the international search 20 April, 2000 (20.04.2000)	Date of mailing of the international search report 27 APR 2000 (27.04.00)	
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